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Respectfully submitted,
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MARKED-UP SPECIFICATION

The paragraph beginning at page 20, lines 12-17 has been amended as follows:

Figure 2

Structure of GST-HD fusion proteins (SEQ ID NO:6)

The amino acid sequence corresponding to exon 1 of huntingtin is boxed. Arrows labeled Xa and T indicate cleavage sites for factor Xa and trypsin, respectively.

The paragraph beginning at page 22, lines 9-14 has been amended as follows:

Figure 8

Structure of GST-HD fusion proteins (SEQ ID NOS:38-41, respectively in order of appearance). The amino acid[s] sequences corresponding to the N-terminal portion of huntingtin [is] are boxed and the amino acids corresponding to the biotinylation site are underlined. Arrows labeled (Xa) and (T) indicate cleavage site for factor Xa and trypsin, respectively.

The paragraph beginning at page 23, line 30 through page 25, line 12 has been amended as follows:

Example 1:

Purification of GST-HD fusion proteins containing expanded polyglns

Exon 1 of the HD gene was isolated from genomic phage clones, derived from the normal and expanded alleles of an HD patient (Sathasivam et al., 1997), and used for the expression of GST-HD fusion proteins in *E. coli*. DNA fragments containing CAG repeats in the normal $(CAG)_{20-33}$ and expanded $(CAG)_{37-130}$ range were cloned into pGEX-5X-1 (Pharmacia), and the resulting plasmids expressing fusion proteins with 20 (GST-HD20), 30 (-HD30), 51 (-HD51), 83 (-HD83) and 122 (-HD122) glutamines, respectively, were used for protein purification. For plasmid construction lambda phage from stock 9197_4 (Sathasivam et al., 1997) were plated to give single plaques which were inoculated into 400 ml cultures of *E. coli* XL1-Blue MRF' (Stratagene) for DNA preparation. The DNA sequence encoding the N-terminal portion of huntingtin (exon 1), including the CAG repeats, was amplified by PCR using the following pair

of primers: ES 25 (TGGGATCCGCATGGCGACCCTGGAAAAGCTGATGAAGG (SEQ ID NO:1)) corresponding to nt315-343 of the HD gene (HDCRG, 1993) and containing a BamHI site (underlined) and ES 26 (GGAGTCGACTCACGGTCGGTGCAGCGGCTCCTCAGC (SEQ ID NO:2)) corresponding to nt516-588 and containing a SalI site (underlined). Conditions for PCR were as described (Mangiarini et al., 1996). Due to instability of the CAG repeat during propagation in *E. coli*, DNA preparations from individual plaques yielded different sized PCR products. Fragments of ~ 320, 360, 480 and 590 bp were gel-purified digested with BamHI and SalI and inserted into the BamHI-SalI site of the expression vector pGEX-5X-1 (Pharmacia), yielding pCAG30, pCAG51, pCAG83 and pCAG122, respectively. pCAG20, containing 20 repeats of CAG within the cloned HD exon 1 sequence, was similarly constructed from a phage genomic clone derived from a normal allele. All constructs were verified by sequencing. After induction with IPTG, the resulting proteins were purified under native conditions by affinity chromatography on glutathione agarose. Thus, *E. coli* SCS1 (Stratagene) carrying the pGEX expression plasmid of interest was grown to an OD_{600nm} of 0.6 and induced with IPTG (1mM) for 3.5h as described in the manufacturer's protocol (Pharmacia). Cultures (200 ml) of induced bacteria were centrifuged at 4000g for 20 min, and the resulting pellets were stored at -80°C. Cells were thawed on ice and resuspended in 5ml of lysis buffer (50mM sodium phosphate, 150mM NaCl, 1mM EDTA, pH 7.4) containing 0.5mg/ml lysozyme. After 45 min at 0°C, cells were sonicated with two 30sec-bursts. Octyl-β-D-glucopyranoside was then added to a final concentration of 0.1% and the resulting lysate was clarified by centrifugation at 30,000g for 30min at 4°C. Cleared lysates were incubated for 1h at 4°C with 500 µl of a 1:1 slurry of glutathione-agarose beads (Sigma) that had been washed times and resuspended in lysis buffer. The beads were poured into a small column and washed extensively with lysis buffer containing 0.1% octyl-β-D0glucopyranoside. The bound fusion protein was eluted with 2ml of 15mM glutathione (reduced) in lysis buffer. Typical yields were 0.5-1mg of purified GST-HD20, -HD30 and –HD51 proteins per 200ml of bacterial culture; yields of GST-HD83 and –HD122 were much lower, less than 10% of that obtained with the shorter fusion proteins. Protein was determined by the Bio-Rad dye binding assay using bovine serum albumin as standard. SDS-PAGE of the purified GST-HD20, -HD30, -HD51, -HD83 and -HD122 proteins revealed major bands of 42, 45, 50, 65 and 75kDa, respectively (Fig. 1a). These bands were also detected when the various protein fractions were subjected to immunoblot analysis using the affinity purified

anti-huntingtin antibody HD1 (Fig. 1b, lanes 2-6). HD1 specifically detects the GST-HD fusion proteins on immunoblots, whereas the GST-tag alone is not recognized (Fig. 1b, lane 1). For immunoblotting a bacterial plasmid encoding HD1-His, a His_6-tagged fusion protein containing residues 1-222 of huntingtin, was generated by inserting a PCR-amplified IT-15 cDNA fragment into the pQE-32 vector (Qiagen). The fusion protein was expressed in *E. coli*, affinity-purified under denaturing conditions on Ni-NTA agarose, and injected into rabbits. The resulting immune serum was then affinity-purified against the antigen that had been immobilized on Ni-NTA agarose. The GAPDH- and Fos B- specific antisera have been described (Wanker et al., 1997; Davies et al., 1997).